What is claimed is:

1. A sterilization indicator for testing the effectiveness of a sterilization procedure, comprising:

(a) a source of an active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(b) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical; and

(c) a substrate that is capable of reacting with the active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure.

2. A sterilization indicator according to claim 1, wherein the source of an active enzyme comprises a microorganism.

3. A sterilization indicator according to claim 2, wherein the source of an active enzyme comprises *Bacillus stearothermophilus* spores.

4. A sterilization indicator according to claim 1, wherein the source of an active enzyme comprises a purified enzyme.

5. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises a surfactant.

6. A sterilization indicator according to claim 5, wherein the sterilant-resistant chemical further comprises a hydrophobic additive.

7. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises a polyglycerol alkyl ester or a polyglycerol alkyl ether.

8. A sterilization indicator according to claim 7, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl monostearate, hexaglyceryl monostearate, tetraglyceryl monostearate, hexaglyceryl polyricinolate, decaglyceryl monolaurate, hexaglyceryl monolaurate, tetraglyceryl monololeate, decaglyceryl trioleate, decaglyceryl monooleate, decaglyceryl dipalmitate, hexaglyceryl distearate, decaglyceryl monooleate, decaglyceryl monomyristate, decaglyceryl monoisostearate, and decaglyceryl diisostearate, and mixtures of two or more members of the group.

9. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises an ethoxylated polyhydric alcohol ester or an ethoxylated polyhydric alcohol ether.

10. A sterilization indicator according to claim 9, wherein the sterilant-resistant chemical is a compound selected from the group consisting of glycereth-7-diisononanoate, polyoxyethylene (5) glyceryl monostearate, and mixtures of two or members of the group.

11. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises decaglycerol.

12. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises decaglyceryl monostearate and sorbitol.

13. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises decaglyceryl monostearate.

14. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises tetraglyceryl monostearate.

15. A sterilization indicator according to claim 1, wherein the sterilant-resistant chemical comprises glycereth-7-diisononanoate.

16. A sterilization indicator for testing the effectiveness of a sterilization procedure, comprising:

(a) a compressible outer container having at least one opening to allow sterilant to enter the outer container during the sterilization procedure;

(b) a source of an active enzyme contained within the outer container, the enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(c) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical; and

(d) a breakable inner container within the outer container that is impermeable to the sterilant used in the sterilization procedure and that contains a substrate, wherein the inner container is adapted so that it may be broken by compressing the outer container, to allow the substrate to contact the enzyme, and wherein the substrate is capable of reacting with active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure.

17. A sterilization indicator according to claim 16, wherein the substrate is contained within the inner container.

18. A sterilization indicator according to claim 16, wherein the source of an active enzyme comprises a microorganism.

19. A sterilization indicator according to claim 18, wherein the source of an active enzyme comprises *Bacillus stearothermophilus* spores.

20. A sterilization indicator according to claim 16, wherein the source of an active enzyme comprises a purified enzyme.

21. A sterilization indicator according to claim 16, wherein the sterilant-resistant chemical comprises a surfactant.

22. A sterilization indicator according to claim 21, wherein the sterilant-resistant chemical further comprises a surfactant and a hydrophobic additive.

23. A sterilization indicator according to claim 16, wherein the sterilant-resistant chemical comprises a polyglycerol alkyl ester or a polyglycerol alkyl ether.

24. A sterilization indicator according to claim 23, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl monostearate, hexaglyceryl monostearate, tetraglyceryl monostearate, hexaglyceryl polyricinolate, decaglyceryl monolaurate, hexaglyceryl monolaurate, tetraglyceryl monolaurate, decaglyceryl trioleate, decaglyceryl monooleate, decaglyceryl dipalmitate, hexaglyceryl distearate, decaglyceryl monooleate, decaglyceryl monomyristate, decaglyceryl monoisostearate, and decaglyceryl diisostearate, and mixtures of two or more members of the group.

25. A sterilization indicator according to claim 16, wherein the sterilant-resistant chemical comprises an ethoxylated polyhydric alcohol ester or an ethoxylated polyhydric alcohol ether.

26. A sterilization indicator according to claim 25, wherein the sterilant-resistant chemical is a compound selected from the group consisting of glycereth-7-diisononanoate, polyoxyethylene (5) glyceryl monostearate, and mixtures of two or members of the group.

27. A sterilization indicator according to claim 16, wherein the sterilant-resistant chemical comprises decaglycerol.

28. A method for testing the effectiveness of a sterilization procedure, comprising the steps of:

(a) providing a source of an active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(b) treating the source of active enzyme with a sterilant-resistant chemical so that the enzyme is more resistant to inactivation by a sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical;

(c) providing a substrate that is capable of reacting with the active enzyme to form an enzyme-modified product to provide a detectable indication of the failure of a sterilization procedure;

(d) subjecting the source of active enzyme that has been treated with a sterilant-resistant chemical to a sterilization procedure;

(e) combining the enzyme and substrate; and

(f) examining the sterilization indicator for a detectable signal.

29. A sterilization indicator for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a source of active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the

effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(b) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a hydrogen peroxide plasma sterilization procedure than it would be if were not associated with the sterilant resistant chemical; and

(c) a substrate that is capable of reacting with the active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure.

30. A sterilization indicator according to claim 29, wherein the source of an active enzyme comprises a microorganism.

31. A sterilization indicator according to claim 30, wherein the source of an active enzyme comprises *Bacillus stearothermophilus* spores.

32. A sterilization indicator according to claim 30, wherein the source of an active enzyme comprises *Bacillus subtilis* spores.

33. A sterilization indicator according to claim 29, wherein the source of an active enzyme is a purified enzyme.

34. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises decaglyceryl decaoleate.

35. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises decaglycerol pentaoleate.

36. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises tetraglycerol monooleate.

37. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises decaglyceryl tri-oleate.

38. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises decaglycerol hexaoleate.

39. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises hexaglycerol dioleate.

40. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises polyoxyethylene (60) glycerol monostearate.

41. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises polyoxyethylene (20) sorbitan monostearate.

42. A sterilization indicator according to claim 29, wherein the sterilant-resistant chemical comprises hexaglyn di-stearate.

43. A sterilization indicator for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a compressible outer container having at least one opening to allow sterilant to enter the outer container during the sterilization procedure;

(b) a source of an active enzyme contained within the outer container, the enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be

substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(c) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a hydrogen peroxide sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical; and

(d) a breakable inner container within the outer container that is impermeable to the sterilant used in the sterilization procedure and that contains a substrate, wherein the inner container is adapted so that it may be broken by compressing the outer container to allow the substrate to contact the enzyme, and wherein the substrate is capable of reacting with active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure.

44. A sterilization indicator according to claim 43, wherein the source of active enzyme comprises a microorganism.

45. A sterilization indicator according to claim 44, wherein the source of active enzyme comprises *Bacillus stearothermpohhilus* spores.

46. A sterilization indicator according to claim 44, wherein the source of active enzyme comprises *Bacillus subtilis* spores.

47. A sterilization indicator according to claim 43, wherein the source of active enzyme comprises a purified enzyme.

48. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises decaglyceryl decaoleate.

49. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises decaglycerol pentaoleate.

50. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises tetraglycerol monooleate.

51. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises decaglyceryl tri-oleate.

52. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises decaglycerol hexaoleate.

53. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises hexaglycerol dioleate.

54. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises polyoxyethylene (60) glycerol monostearate.

55. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises polyoxyethylene (20) sorbitan monostearate.

56. A sterilization indicator according to claim 43, wherein the sterilant-resistant chemical comprises hexaglyn di-stearate.

57. A non-challenge test pack for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a thermally-resistant plastic tray for holding a sterilization indicator, said tray comprising an elevated rim surface defining the perimeter of the tray and a recessed trough for receiving a sterilization indicator, said rim surface including a plurality of spaced-apart grooves along its length extending through the rim to the recessed trough;

(b) a sterilization indicator within the recessed trough of the tray, for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, the sterilization indicator comprising:

(i) a source of active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(ii) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a hydrogen peroxide plasma sterilization procedure than it would be if it were not associated with the sterilant resistant chemical; and

(iii) a substrate that is capable of reacting with the active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of the sterilization procedure; and

(c) a thermally-resistant plastic lid associated with the rim surface of the plastic tray, said lid forming a substantially sterilant-impermeable seal with the rim surface, and said lid forming a plurality of channels with the grooves in the tray, such that sterilant may enter the tray through the channels during a sterilization procedure and contact the sterilization indicator, wherein the enzyme in the sterilization indicator in the test pack is no more resistant to inactivation by a hydrogen peroxide plasma sterilization procedure than it would be if it were exposed to the procedure without the test pack.

58. A non-challenge test pack according to claim 57, wherein the source of an active enzyme is a microorganism.

59. A non-challenge test pack according to claim 58, wherein the microorganism is *Bacillus stearothermophilus* spores.

60. A non-challenge test pack according to claim 58, wherein the microorganism is *Bacillus subtilis.*

61. A non-challenge test pack according to claim 57, wherein the source of active enzyme is a purified enzyme.

62. A sterilization test pack according to claim 57, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl decaoleate, decaglycerol pentaoleate, tetraglycerol monooleate, decaglyceryl tri-oleate, decaglycerol hexaoleate, hexaglycerol dioleate, polyoxyethylene (60) glycerol monostearate, polyoxyethylene (20) sorbiatan monostearate, and hexaglyn di-stearate, and mixtures of two or more members of the group.

63. A non-challenge test pack for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a thermally-resistant plastic tray for holding a sterilization indicator, said tray comprising an elevated rim surface defining the perimeter of the tray and a recessed trough for receiving a sterilization indicator, said rim surface including a plurality of spaced-apart grooves along its length extending through the rim to the recessed trough;

(b) a sterilization indicator within the recessed trough of the tray, for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, the sterilization indicator comprising:

(i) a compressible outer container having at least one opening to allow sterilant to enter the outer container during the sterilization procedure;

(ii) a source of an active enzyme contained within the outer container, the enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(iii) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to

inactivation by a hydrogen peroxide sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical; and

(iv) a breakable inner container within the outer container that is impermeable to the sterilant used in the sterilization procedure and that contains a substrate, wherein the inner container is adapted so that it may be broken by compressing the outer container to allow the substrate to contact the enzyme, and wherein the substrate is capable of reacting with active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure; and

(c) a thermally-resistant plastic lid associated with the rim surface of the plastic tray, said lid forming a substantially sterilant-impermeable seal with the rim surface, and said lid forming a plurality of channels with the grooves in the tray, such that sterilant may enter the tray through the channels and contact the sterilization indicator, wherein the enzyme in the sterilization indicator in the test pack is no more resistant to inactivation by a hydrogen peroxide plasma sterilization procedure than it would be if it were exposed to the procedure without the test pack.

64. A non-challenge test pack according to claim 63, wherein the source of an active enzyme is a microorganism.

65. A non-challenge test pack according to claim 64, wherein the microorganism is *Bacillus stearothermophilus* spores.

66. A non-challenge test pack according to claim 64, wherein the microorganism is *Bacillus subtilis* spores.

67. A non-challenge test pack according to claim 63, wherein the source of an active enzyme is a purified enzyme.

68. A sterilization test pack according to claim 63, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl

decaoleate, decaglycerol pentaoleate, tetraglycerol monooleate, decaglyceryl tri-oleate, decaglycerol hexaoleate, hexaglycerol dioleate, polyoxyethylene (60) glycerol monostearate, polyoxyethylene (20) sorbiatan monostearate, and hexaglyn di-stearate, and mixtures of two or more members of the group.

69. A lumen-challenge test pack for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a thermally-resistant plastic tray for holding a sterilization indicator, said tray comprising a substantially planar surface, an edge defining the outer perimeter of the tray, a recessed trough for receiving a sterilization indicator, and a recessed groove of a defined length and cross-sectional area extending through the recessed trough and penetrating the edge of the tray at two points;

(b) a sterilization indicator within the recessed trough of the tray, for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, the sterilization indicator comprising:

(i) a source of active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(ii) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a hydrogen peroxide plasma sterilization procedure than it would be if it were not associated with the sterilant resistant chemical; and

(iii) a substrate that is capable of reacting with the active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of the sterilization procedure; and

(c) a thermally-resistant plastic lid associated with the tray, said lid forming a substantially sterilant-impermeable seal with the planar surface of the tray and

forming a lumen path with the recessed groove in the tray, said lumen path having the defined length and cross-sectional surface area of the recessed groove, wherein sterilant may enter the test pack at either end of the lumen path during a sterilization procedure and contact the sterilization indicator.

70. A lumen-challenge test pack according to claim 69, wherein the source of an active enzyme is a microorganism.

71. A lumen-challenge test pack according to claim 70, wherein the microorganism is *Bacillus stearothermophilus* spores.

72. A lumen-challenge test pack according to claim 70, wherein the microorganism is *Bacillus subtilis* spores.

73. A lumen-challenge test pack according to claim 69, wherein the source of an active enzyme is a purified enzyme.

74. A lumen-challenge test pack according to claim 69, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl decaoleate, decaglycerol pentaoleate, tetraglycerol monooleate, decaglyceryl tri-oleate, decaglycerol hexaoleate, hexaglycerol dioleate, polyoxyethylene (60) glycerol monostearate, polyoxyethylene (20) sorbitan monostearate, and hexaglyn di-stearate, and mixtures of two or more members of the group.

75. A lumen-challenge test pack according to claim 69, wherein the recessed trough is located at the approximate midpoint of the defined length of the lumen path.

76. A lumen-challenge test pack according to claim 69, wherein the defined length of the lumen path is approximately 12 inches (30.48 cm) and the defined cross-sectional area of the lumen path is approximately the cross-sectional area of a circle having a diameter of .25 inches (.635 cm).

77. A lumen-challenge test pack for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, comprising:

(a) a thermally-resistant plastic tray for holding a sterilization indicator, said tray comprising a substantially planar surface, an edge defining the outer perimeter of the tray, a recessed trough for receiving a sterilization indicator, and a recessed groove of a defined length and cross-sectional area extending through the recessed trough and penetrating the edge of the tray at two points;

(b) a sterilization indicator within the recessed trough of the tray, for testing the effectiveness of a hydrogen peroxide plasma sterilization procedure, the sterilization indicator comprising:

(i) a compressible outer container having at least one opening to allow sterilant to enter the outer container during the sterilization procedure;

(ii) a source of an active enzyme contained within the outer container, the enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a sterilization procedure, wherein the enzyme is substantially inactivated by a sterilization procedure that is lethal to the test microorganism, but wherein the enzyme may not be substantially inactivated by a sterilization procedure that is sublethal to the test microorganism;

(iii) a sterilant-resistant chemical associated with the source of active enzyme in such a manner that the active enzyme is more resistant to inactivation by a hydrogen peroxide sterilization procedure than it would be if it were not associated with the sterilant-resistant chemical; and

(iv) a breakable inner container within the outer container that is impermeable to the sterilant used in the sterilization procedure and that contains a substrate, wherein the inner container is adapted so that it may be broken by compressing the outer container to allow the substrate to contact the enzyme, and wherein the substrate is capable of reacting with active enzyme to form an enzyme-modified product that provides a detectable indication of the failure of a sterilization procedure; and

(c) a thermally-resistant plastic lid associated with the tray, said lid forming a substantially sterilant-impermeable seal with the planar surface of the tray and forming a lumen path with the recessed groove in the tray, said lumen path having the defined length and cross-sectional surface area of the recessed groove, wherein sterilant may enter the test pack at either end of the lumen path during a sterilization procedure and contact the sterilization indicator.

78. A lumen-challenge test pack according to claim 77, wherein the source of an active enzyme is a microorganism.

79. A lumen-challenge test pack according to claim 78, wherein the microorganism is *Bacillus stearothermophilus* spores.

80. A lumen-challenge test pack according to claim 78, wherein the microorganism is *Bacillus subtilis* spores.

81. A lumen-challenge test pack according to claim 77, wherein the source of an active enzyme is a purified enzyme.

82. A lumen-challenge test pack according to claim 77, wherein the sterilant-resistant chemical is a compound selected from the group consisting of decaglyceryl decaoleate, decaglycerol pentaoleate, tetraglycerol monooleate, decaglyceryl tri-oleate, decaglycerol hexaoleate, hexaglycerol dioleate, POE (60) glycerol monostearate, POE (20) sorbitan monostearate, and hexaglyn di-stearate, and mixtures of two or more members of the group.

83. A lumen-challenge test pack according to claim 77, wherein the recessed trough is located at the approximate midpoint of the defined length of the lumen path.

84. A lumen-challenge test pack according to claim 77, wherein the defined length of the lumen path is approximately 12 inches (30.48 cm) and the defined cross-

sectional area of the lumen path is approximately the cross-sectional area of a circle having a diameter of .25 inches (.635 cm).

85. A method for stabilizing active enzymes in the presence of heat, the method comprising:

(a) providing a source of an active enzyme;

(b) treating the source of active enzyme with a sterilant-resistant chemical that causes the enzyme to be more resistant to inactivation by heat than it would be if the sterilant-resistant chemical were not present;

(c) providing a substrate that reacts with the active enzyme to form an enzyme-modified product;

(d) subjecting the source of active enzyme that has been treated with a sterilant-resistant chemical to heat;

(e) combining the active enzyme and the substrate;

(f) determining whether the enzyme-modified product, which has a detectable signal, is present or absent; and

(g) correlating the presence of the detectable signal with success of the steriliant-resistant chemical in stabilizing the active enzyme and the absence of the detectable signal with failure of the steriliant-resistant chemical to stabilize the active enzyme.

86. The method according to claim 28, further comprising:

(h) subjecting a test pack, a sterilization indicator which has been treated with a variable amount of sterilant-resistant chemical to serve as a control for monitoring the effectiveness of a steam sterilization procedure, to a steam sterilization procedure.

87. The method according to claim 85, further comprising:

(h) subjecting a test pack, a sterilization indicator which has been treated with a variable amount of sterilant-resistant chemical to serve as a control for monitoring the effectiveness of the sterilant-resistant chemical in stabilizing the active enzyme, to the second higher temperature.

88. A method for testing the effectiveness of a steam sterilization procedure, the method comprising:

(a) providing a source of an active enzyme having an enzyme activity that is correlated with the survival of at least one test microorganism commonly used to monitor the effectiveness of a steam sterilization procedure, wherein the enzyme is substantially inactivated by a steam sterilization procedure that is lethal to the test microorganism, but wherein the enzyme is not substantially inactivated by a steam sterilization procedure that is sublethal to the test microorganism;

(b) treating the source of active enzyme with a sterilant-resistant chemical comprising a surfactant and a hydrophobic additive selected from the group consisting of short chain alkyl or aryl esters (C1-C6) of long chain (straight or branched) alkyl or alkenyl alcohols or acids (C8-C36) and their polyethoxylated derivatives; short chain alkyl or aryl esters (C1-C6) of C4-C12 diacids or diols, optionally substituted in available positions by -OH; alkyl or aryl C1-C9 esters of glycerol, pentaerythritol, ethylene glycol; C12-C22 alkyl ester or ethers of polypropylene glycol; C12-C22 alkyl esters or ethers of polypropylene glycol/polyethylene glycol copolymer; poly ether polysiloxane copolymers; cyclic dmethicones; polydialkylsiloxanes; polyaryl/alkylsiloxanes; long chain (C8-C36) alkyl and alkenyl esters of long straight or branched chain alkyl or alkenyl alcohols or acids; long chain (C8-C36) alkyl of alkenyl amides of long straight or branched chain (C8-C36) alkyl or alkenyl amines or acids; hydrocarbons including straight and branched chain alkanes and alkenes; polysiloxane polyalkylene copolymers; dialkoxy dimethyl polysiloxanes; short chain alkyl or aryl esters (C1-C6) of C12-C22 diacids or diols, optionally substituted in available positions by OH; and C12-C22 alkyl and alkenyl alcohols; isostearyl alcohol; cetyl alcohol; diisopropyl

adipate; squalene; squalane; polyethylene waxes; mineral oil; isopropyl myristate; dimethicone; lanolin; petrolatum; and combinations thereof;

(c) providing a substrate that reacts with the active enzyme to form an enzyme modified product, which has a detectable signal, upon failure of a steam sterilization procedure;

(d) subjecting the source of active enzyme that has been treated with a sterilant-resistant chemical to a steam sterilization procedure;

(e) combining the enzyme and the substrate;

(f) determining whether the enzyme-modified product, which has a detectable signal, is present or absent; and

(g) correlating the presence of the detectable signal with failure of the steam sterilization procedure and the absence of the detectable signal with success of the steam sterilization procedure.